

Monocytotropic Human Immunodeficiency Virus Type 1 (HIV-1) Variants Detectable in All Stages of HIV-1 Infection Lack T-Cell Line Tropism and Syncytium-Inducing Ability in Primary T-Cell Culture

HANNEKE SCHUITEMAKER,¹ NEELTJE A. KOOTSTRA,¹ RUUD E. Y. DE GOEDE,¹
FRANK DE WOLF,^{2†} FRANK MIEDEMA,¹ AND MATTHIJS TERSMETTE^{1*}

Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology of the University of Amsterdam¹ and Municipal Health Center,² Amsterdam, The Netherlands

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We previously demonstrated a correlation between the presence of syncytium-inducing (SI) human immunodeficiency virus type 1 (HIV-1) variants showing tropism for cell line H9 and the occurrence of rapid CD4 cell decline and progression to AIDS. In contrast, in stable asymptomatic individuals, we detected only isolates with low replication rates that were non-syncytium-inducing (NSI) and nontropic for the H9 cell line. Here, we investigated the monocytotropism of established HIV-1 isolates with a panel of isolates and with biological HIV-1 clones with distinct phenotypes. Moreover, the prevalence and biological phenotypes of monocytotropic HIV-1 variants in the course of HIV-1 infection were analyzed in comparative primary isolation studies on peripheral blood lymphocytes (PBL) and monocyte-derived macrophages (MDM). In cell-free infection studies with MDM from eight blood donors, 13 of 17 NSI isolates but only 4 of 14 SI isolates were able to infect MDM. NSI isolates also infected significantly more different donors than SI variants (median, 3 of 8 versus 0 of 8). This enhanced monocytotropism of NSI isolates was confirmed in experiments with biological HIV-1 clones with distinct phenotypes recovered from the same donor. To investigate the prevalence and biological phenotypes of monocytotropic variants in different stages of HIV-1 infection, sequential isolates from peripheral blood mononuclear cell samples from nine asymptomatic individuals, five of whom progressed to AIDS and seven of whom had a known time of seroconversion, were recovered by cocultivation with both PBL and MDM. Monocytotropic variants were obtained from 37 of 42 time points. All monocytotropic variants were NSI in PBL culture and non-T-cell-line tropic, even when SI, T-cell-line-tropic HIV-1 variants could be recovered from the same patient sample by cocultivation with PBL. We conclude that monocytotropic HIV-1 variants mostly have an NSI phenotype in PBL and, in contrast to SI variants, are present at all stages of HIV-1 infection. These results suggest an important role for monocytotropic variants in the persistence of HIV-1 infection.

In studies on human immunodeficiency virus type 1 (HIV-1) isolates obtained from peripheral blood mononuclear cell (PBMC) samples from infected individuals by coculture with phytohemagglutinin (PHA)-stimulated donor peripheral blood lymphocytes (PBL), we and others have observed differences in viral biological properties, such as syncytium-inducing (SI) capacity, replication rate, and tropism for particular cell lines (1, 4, 9, 32, 39). In longitudinal studies, we have demonstrated an association between the clinical course of infected individuals and the biological phenotype of their sequential HIV-1 isolates (33). Recovery of SI, H9-cell-line-tropic variants was correlated with subsequent rapid progression to AIDS (33, 34). The SI variants appeared only in the course of infection, preceding clinical deterioration (33). In long-term asymptomatic individuals, non-syncytium-inducing (NSI) variants were detected from the time of seroconversion on (33). These NSI HIV-1 variants were unable to replicate in the H9 cell line and showed variable replication rates. Furthermore, a correlation between the replication rate and the rate of CD4⁺ cell decline in vivo could be demonstrated (33).

Besides CD4⁺ T cells, cells of the monocyte/macrophage lineage are a major target for HIV-1 (14, 15, 23). These cells may become persistently infected and may serve as a virus reservoir, disseminating the virus throughout the body (21, 41). In previous studies, differences in monocytotropism among HIV-1 isolates, reminiscent of the biological variability described above, have been documented (5, 13, 14, 16, 23, 28). To further elucidate the pathogenic significance of monocytotropic HIV-1 variants, we tested a panel of isolates and biological clones with distinct biological phenotypes, originally obtained by coculture with PBL, for their ability to replicate in monocyte-derived macrophages (MDM) from different donors. Furthermore, in primary isolation studies we compared the biological properties of sequential isolates obtained by coculture with either PBL or MDM from the same patient PBMC samples. In these studies we demonstrate that monocytotropic HIV-1 isolates are present in the peripheral blood throughout the course of HIV-1 infection and that the majority of these isolates have an NSI, non-T-cell-line-tropic phenotype.

MATERIALS AND METHODS

Cell isolation and culture. Monocytes, >95% pure, were prepared from PBMC from HIV-1 antibody-seronegative plasmapheresis donors as described previously (10) and

* Corresponding author.

† Present address: Department of Virology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands.

cultured *in vitro*, which resulted in MDM. In brief, PBMC were obtained from citrated venous blood by isolation on a Percoll density gradient and then further enriched for monocytes by centrifugal elutriation. [³H]thymidine incorporation in these cells upon stimulation with phytohemagglutinin (PHA) did not exceed background levels, demonstrating the virtual absence of T cells in the monocyte preparation. Monocytes were cultured for 5 days at a cell concentration of 10⁶ monocytes per ml in endotoxin-free (22) Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% pooled human serum, penicillin (100 U/ml), streptomycin (100 µg/ml), and, in some cases, granulocyte-macrophage colony-stimulating factor (GM-CSF; 100 U/ml; Sandoz, Basel, Switzerland). Cells were plated in 24-well plastic tissue culture plates (Nunc) at 1 ml per well and maintained at 37°C in a humidified atmosphere supplemented with 5% CO₂. Cultures were kept for 6 to 7 weeks, and the medium was changed every fifth day during the first 25 days of culture and once a week from then on.

PBL. PBMC were suspended at 5 × 10⁶ cells per ml in IMDM supplemented with 10% fetal calf serum (Hyclone), penicillin (100 U/ml), streptomycin (100 µg/ml), and PHA (5 µg/ml). After 2 days, the nonadherent cells were harvested. These PBL were infected and resuspended to a cell concentration of 10⁶/ml in 8 ml of medium (without PHA) supplemented with partially purified interleukin-2. Medium was changed twice a week, and once a week fresh 2-day PHA-stimulated PBL from a seronegative blood donor were added to the cultures.

H9 and Sup-T1 cell lines. H9 and Sup-T1, both CD4⁺ T lymphocyte cell lines, were maintained at 5 × 10⁵ cells per ml in IMDM with 10% newborn calf serum (GIBCO), penicillin (100 U/ml), and streptomycin (100 µg/ml).

Viruses. For cell-free infection of MDM or PBL, a panel of established virus isolates (32–34), 17 NSI and 14 SI, was used. SI variants typically induce one to five syncytia per 100× field in PBL culture. In contrast, syncytia were absent (<1 syncytium per 10 100× fields) in PBL cultures infected with NSI variants (32). Except for the monocyotropic virus HTLV-III Ba-L (a kind gift of M. Popovic, New Mexico State University Primate Research Institute, Alamogordo, N.Mex.), originally obtained from alveolar macrophages which displayed an NSI phenotype in PBL culture, and the T-cell-line-tropic virus HTLV-IIIB (29), with an SI phenotype in PBL culture, all variants used were obtained by coculture of PBMC from HIV-1 antibody-seropositive individuals with PBL stimulated with PHA and interleukin-2 as described previously (32, 33). In addition to these virus isolates from bulk cultures, seven biological clones, four SI and three NSI, were used, recovered from two sequentially obtained blood samples from an asymptomatic seropositive individual by direct limiting-dilution HIV-1 isolation (unpublished data). Patient cells (10,000 per well) were cocultivated with donor PBL (100,000 per well) in 96-well microtiter plates. Culture conditions were similar to those described for bulk culture (32). Virus replication was detected by a p24 capture assay of the culture supernatants. Positive cultures were expanded for biological phenotyping and stock preparation. The number of HIV-1-producing patient cells per well was estimated with the formula $\mu = -\ln F_0$ (Poisson distribution), in which F_0 is the fraction of negative cultures per total number of cultures (24). Virus stocks were grown in PHA-stimulated PBL except for HTLV-III Ba-L, which was grown on monocytes, and cell-free supernatants were frozen at –80°C. Virus numbers in stocks were determined by

measuring reverse transcriptase (RT) activity as described before (32).

Studies with established HIV-1 isolates. After 5 days of culture, MDM were infected with cell-free inocula (5 × 10⁵ cpm of RT activity) of the HIV-1 isolates or biological clones described above. After 48 h, unadsorbed virus and residual nonadherent cells were removed by washing each well four times with a total volume of 4 ml of medium. Cells were refed with 1 ml of IMDM with 10% heat-inactivated pooled human serum, penicillin, and streptomycin. Virus production was tested each week in a p24 antigen capture enzyme-linked immunosorbent assay (ELISA).

Two-day PHA-stimulated PBL (8 × 10⁶ cells) were infected in a volume of 0.5 ml with a virus inoculum with an RT activity of 5 × 10⁵ cpm. After 2 h of incubation at 37°C, cells were washed and further cultured in 8 ml of PBL medium.

Cell lines H9 and Sup-T1 were infected by the same protocol.

In PBL and cell line cultures, virus production was tested twice a week in a p24 antigen capture ELISA.

Subjects. Primary virus isolation studies were performed on blood samples obtained from homosexual men participating in a cohort study in Amsterdam, The Netherlands (8). All subjects were seropositive and asymptomatic (Centers for Disease Control stage II or III) at the beginning of the observation period. Three patients (no. 105, 232, and 316) received zidovudine in the course of the period of observation (7).

Virus isolation from PBMC of HIV-1-infected individuals. Virus isolation from PBMC of seropositive HIV-1-infected individuals was performed by cocultivation with MDM cultured for 5 days. All cultures were performed in duplicate, with MDM precultured in the absence or presence of 100 U of GM-CSF per ml. On day 5 of MDM culture, aliquots of PBMC (10⁶ cells) were admixed with equal numbers of adherent MDM in 16-mm-diameter culture wells. Medium was changed every week, and the supernatant was stored at –80°C. A sample of each supernatant was taken to test for virus production. Positive samples were subsequently passaged to PBL. After PBL passage, transmission to the T-cell lines H9 and Sup-T1 was performed by cell-free transmission and cocultivation.

Virus detection. Virus production was assessed in a p24 antigen capture ELISA (36). Triton X-100-treated culture supernatant samples or Triton X-100-lysed cells were added to microtiter plates (Nunc) coated with purified, pooled human anti-HIV-1 immunoglobulin G (30). Bound p24 was detected with horseradish peroxidase-labeled monoclonal antibody to p24, shown to recognize all HIV-1 isolates (36). A culture was considered positive when p24 levels were higher than two times the level in the negative control in at least two successive supernatant samples. The absence of p24 in supernatant samples negative in the in-house p24 capture assay (cutoff, 300 pg/ml) was confirmed in a more sensitive commercial p24 antigen capture assay (Abbott Laboratories, Chicago, Ill.).

Three times a week, the PBL cultures were checked for syncytium formation as described before (32). Each time, at least 10 microscope fields (magnification, ×100) were observed.

RESULTS

Replication of established HIV-1 isolates in MDM and PBL. We compared two groups of established NSI ($n = 17$) and SI

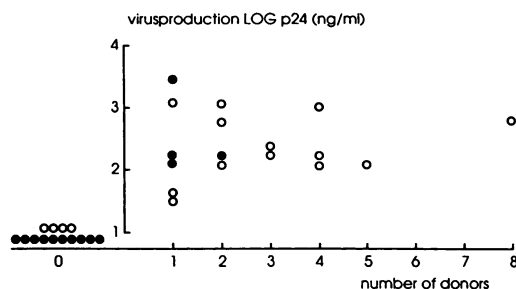


FIG. 1. Ability of NSI and SI variants to establish productive infection in MDM from eight different donors. Five-day-cultured MDM were infected with cell-free virus (inoculum, 5×10^5 cpm of RT activity) originally obtained by coculture of PBMC from seropositive individuals with PHA-stimulated PBL. Virus production in MDM cultures was measured repeatedly in a p24 antigen capture ELISA. Cultures were considered positive when at least two sequential samples gave values more than two times the background extinction value. For each virus, the number of donors whose MDM could be infected and the mean peak p24 production are indicated. Symbols: ○, NSI isolates; ●, SI isolates.

($n = 14$) isolates for their ability to productively infect MDM and PHA-stimulated PBL cultures. Virus inocula were standardized by RT activity; duplicate MDM cultures were precultured for 5 days in the presence or absence of GM-CSF from eight different blood donors, and PBL cultures were infected with a fixed amount of virus stock (5×10^5 cpm of RT activity). Following infection, culture supernatants were periodically tested for the presence of p24 antigen in a p24 antigen capture ELISA.

The two groups of virus isolates showed a significant difference (Fisher's exact test; $P = 0.013$) in their capacity to productively infect MDM (Fig. 1). Both groups were also significantly different in the number of different donor MDM they could infect (Mann-Whitney U-Wilcoxon rank sum W test; $P = 0.027$). Most NSI isolates (13 of 17) were able to productively infect MDM, and the majority of these isolates could infect monocytes from more than one donor (median, three of eight). Interestingly, all four NSI isolates obtained from patients progressing toward AIDS dementia (34) were able to infect MDM from at least three donors. The prototype monocyctotropic variant HTLV-III Ba-L infected MDM from all eight blood donors. Of 14 SI isolates, however, only 4 could infect MDM and only 1 of these isolates could infect monocytes from more than one donor (median, zero of eight). Variable results in the monocyctotropism of HTLV-III B, a T-cell-line-tropic, SI HIV-1 isolate, have been reported (6, 25, 27, 31). In our laboratory, HTLV-III B was able to infect MDM from one of eight donors.

All the virus isolates used were able to infect 2-day PHA-stimulated PBL (PHA-PBL). The two groups of isolates, NSI and SI, differed in the total amount of virus production and the time lapse between infection and first detection of virus in the culture supernatant (32, 33). Replication of four viruses in both MDM and PBL cultures is shown in Fig. 2. In PHA-PBL, the SI variants ACH-158.15 and ACH-479.8 had the highest replication rate and showed the highest virus production in their group (Fig. 2A). In MDM, only ACH-479.8 was able to replicate, and the replication rate and virus production were low (Fig. 2B). ACH-158.15 was not able to establish a productive infection in MDM at all. For the two NSI isolates, the opposite phenomena were observed: in MDM culture, ACH-63.11

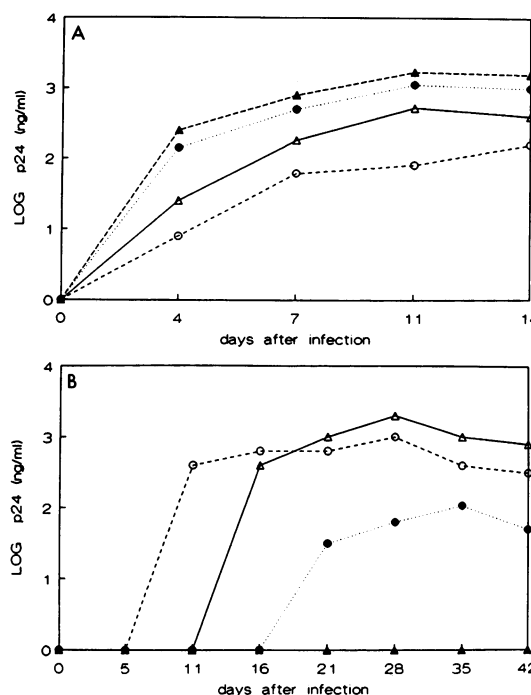


FIG. 2. Time course of virus production in PHA-stimulated PBL cultures (A) and MDM cultures (B) infected with SI variants ACH-158.15 and ACH-479.8 or infected with NSI variants ACH-63.11 and HIV-Ams-101. PHA-stimulated PBL and 5-day-cultured MDM were cell-free infected with the variants originally obtained from PBMC of seropositive individuals by coculture with PHA-stimulated PBL. Aliquots of the culture supernatant were harvested at the times indicated and analyzed for virus production in a p24 antigen capture ELISA. Symbols: △, ACH-63.11; ○, HIV-Ams-101; ●, ACH-479.8; ▲, ACH-158.15.

and HIV-Ams-101 were the earliest detectable isolates and they showed the highest virus production of all the peripheral blood NSI isolates. In PBL culture, however, these isolates appeared to be slow and they replicated to lower titers than the SI variants.

Since intracellular accumulation of virus particles in MDM has been described elsewhere (16, 26), we compared the amount of p24 present intracellularly and in the culture supernatants of 42 infected MDM cultures. p24 antigen could be demonstrated in the cell lysate of all cultures, with p24 detectable in the supernatant. Cultures of two isolates, both of the NSI phenotype, contained no detectable p24 in the supernatant, but were positive for intracellular p24. Intracellular p24 was not detected in any of the cultures inoculated with SI isolates that remained negative for supernatant p24.

TABLE 1. GM-CSF treatment does not enhance the ability of HIV-1 variants to establish productive infection in MDM

Virus produced	No. of cultures			
	NSI isolates		SI isolates	
	Cultured with GM-CSF	Cultured without GM-CSF	Cultured with GM-CSF	Cultured without GM-CSF
Yes	34	40	5	4
No	102	96	107	108

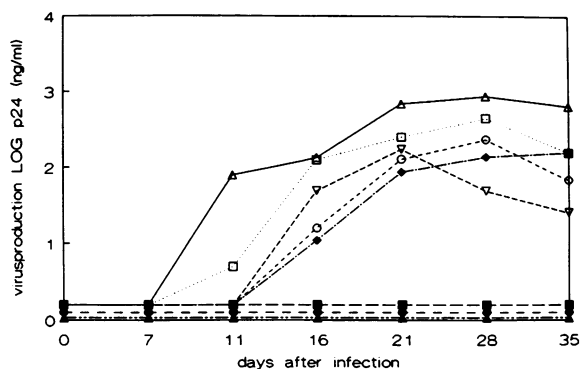


FIG. 3. Time course of virus production in MDM cultures infected with biological clones obtained at two time points from a seropositive individual at the time of SI conversion. Five-day-cultured MDM were cell-free infected with the biological clones originally obtained by coculture with PHA-PBL in a microtiter plate isolation assay. Also, the bulk isolate obtained 12 months before SI conversion was used for cell-free infection. Aliquots of the culture supernatant were harvested at the times indicated and analyzed for virus production in a p24 antigen capture ELISA. Symbols: Δ , ACH-373.21; \circ , ACH-373.38.3; ∇ , ACH-373.38.4; \square , ACH-373.41.3; \blacklozenge , ACH-373.41.1; \blacktriangle , ACH-373.38.1; \bullet , ACH-373.38.2; \blacksquare , ACH-373.41.2.

In a number of cultures infected with SI isolates, repeated rescue of virus was attempted by cocultivation with PHA-PBL at day 2, 4, 7, 14, 21, and 28 following infection. Only from those cultures with detectable p24 in the supernatant could virus be rescued. All of these SI isolates had retained the capacity to induce syncytia in PBL culture. In none of the p24-negative cultures was rescue of virus by cocultivation with PHA-PBL successful.

Since a variation in the level of replication of HIV-1 isolates in PHA-stimulated PBL from different individuals has been reported previously (12, 32), we investigated the extent to which the monocytopathism of isolates is influenced by host-determined differences in susceptibility of MDM to HIV-1 infection. Among MDM from different donors, considerable variability in susceptibility to viral infection was observed. The percentage of NSI isolates capable of establishing a productive infection in MDM from a certain donor ranged from 5.5 to 71%. Only MDM from two donors that were susceptible to infection by most NSI isolates (45 and 71%, respectively) could be infected by SI variants. MDM from the same donor obtained at different

time points displayed similar susceptibilities to HIV-1 isolates, excluding variation between experiments as a cause of the differences observed among donors.

Pretreatment of MDM with GM-CSF overall did not enhance the capacity of either SI or NSI variants to infect MDM (Table 1). Among donors, regardless of the virus phenotype, sometimes more efficient infection of monocytes was observed in either the presence or absence of GM-CSF (not shown), again indicating the influence of host-determined differences in MDM susceptibility.

Infection of MDM by biological HIV-1 clones. Earlier studies demonstrated the existence of multiple, closely related HIV-1 clones within one isolate (11, 39). Since it is likely that, during *in vitro* passage, selection for fast-replicating clones occurs, the isolates used in the studies described above probably do not represent the full spectrum of HIV-1 clones present in the peripheral blood of the individual at the moment of sampling.

In an earlier study, we circumvented the problem of *in vitro* selection of high-replication HIV-1 clones by isolating virus by a direct clonal method using 96-well microtiter plates (35). Biological clones were obtained from two sequential PBMC samples from an asymptomatic seropositive individual (patient 373) at the time of conversion of virus phenotype from NSI to SI. The numbers of positive cultures from the first and second samples were 20 of 96 and 44 of 96, respectively, indicating that the obtained isolates were clonal or oligoclonal at most. The estimated frequencies of HIV-1-producing patient cells of infected cells were 23 per 10^6 and 61 per 10^6 , respectively; these frequencies are similar to frequencies in asymptomatic individuals reported by others (18). From these two time points, seven clones, four with the SI phenotype and three with the NSI phenotype, were tested for their ability to establish a productive infection in MDM. In all cultures infected with NSI clones, virus production was detected. A bulk isolate with the NSI phenotype, obtained 12 months before SI conversion, was also able to infect the MDM culture. In contrast, only one of four cultures inoculated with SI clones showed productive infection. The replication kinetics of the monocytopathic biological clones were comparable to the kinetics of the bulk isolate (Fig. 3).

Comparative primary HIV-1 isolation on PBL and MDM. To analyze the prevalence and the biological phenotype of monocytopathic HIV-1 variants in the course of HIV-1 infection, we isolated virus from patient PBMC by using either MDM or PBL as target cells. Sequential PBMC samples from nine asymptomatic individuals, five of whom

TABLE 2. Primary isolation of HIV-1 from sequential PBMC samples of seropositive individuals by cocultivation with MDM

Patient ^a	Period of clinical follow-up (mo)	Clinical status		No. of samples with successful virus isolation on primary MDM/total no. of samples	Virus phenotype on passage to PBL
		Start ^b	End		
105	19	AS	AIDS	6/6	NSI/SI ^c
186	11	SC	AS	2/2	NSI
232	33	SC	AS	7/7	NSI
316	45	SC	AIDS	7/8	NSI
331	34	SC	AS	2/2	NSI
349	33	AS	AS	4/4	NSI
571	29	SC	AIDS	3/5	NSI
1082	33	SC	AIDS	4/4	NSI
1145	23	SC	AIDS	2/4	NSI

^a All isolates were successfully passaged from MDM to PBL culture. Virus isolation by cocultivation with PBL yielded NSI variants at all time points for patients 186, 232, 331, 349, and 1145 (not shown).

^b AS, Asymptomatic; SC, seroconversion.

^c See Table 3.

TABLE 3. Cocultivation of patient cells with MDM results in preferential isolation of NSI HIV-1 variants even when SI variants can be detected by cocultivation with PBL

Patient	Date of sample (mo/yr)	Virus isolation by PHA-PBL cocultivation			Virus isolation by MDM cocultivation			
		First day of detection	Syncytium induction in PBL	Transmission to cell lines ^a	First day of detection	Syncytium induction in PBL ^b	Transmission to cell lines	Retransmission to MDM ^c
105	08/86	3	+	+	13	—	—	+
	04/87	7	+	+	13	—	—	+
	05/87	7	+	+	13	—	—	+
	07/87	4	+	+	13	—	—	+
	10/87	7	+	+	13	—	—	+
	03/88	4	+	+	7	(+) ^d	—	+
316	06/86 ^e	15	—	—	13	—	—	+
	04/87	7	—	—	13	—	—	+
	05/87	11	—	—	13	—	—	+
	07/87	7	—	—	13	—	—	+
	10/87	7	—	—	13	—	—	+
	03/88	7	+	+	13	—	—	+
	03/89	7	+	+	13	—	—	+
571	06/86 ^e	—	—	—	21	—	—	+
	02/87	—	—	—	—	—	—	—
	08/87	15	—	—	—	—	—	—
	05/88	8	—	—	14	—	—	+
	11/88	8	+	+	14	—	—	+
1082	03/86 ^e	—	—	—	7	—	—	+
	06/86	11	—	—	7	—	—	+
	01/87	8	+	+	14	—	—	+
	10/87	6	+	+	7	—	—	+

^a Transmission to CD4⁺ T-cell lines H9 and SUP-T1.^b Cell-free virus transmission.^c Retransmission after one PBL passage.^d (+), Few, small syncytia.^e Moment of seroconversion.

progressed to AIDS, were used. For seven individuals, the moment of seroconversion was known. For six of these seven individuals, the first isolate was obtained at the time of seroconversion. The first isolate from patient 316 was from a sample obtained 1 year after seroconversion (Table 2). Virus was isolated at 37 of 42 time points tested. The phenotype of these isolates was further investigated by cell-free passages in PBL. Monocytotropism was confirmed by cell-free transmission back to MDM. In all cases (except for the last isolate obtained from patient 105), isolates recovered by cocultivation with MDM were NSI in PBL (Table 2).

The phenotypes of these isolates were compared with the phenotypes of HIV-1 isolates obtained from the same samples by cocultivation with PHA-stimulated PBL. For five patients (no. 186, 232, 331, 349, and 1145), only NSI, non-H9-tropic isolates were obtained in this way (data not shown). For three individuals, conversion of HIV-1 phenotype from NSI and non-T-cell-line-tropic to SI and T-cell-line-tropic (33) was observed in sequential isolates obtained by cocultivation with PBL. In addition, all isolates obtained by PBL cocultivation from subject 105 were of the SI phenotype. In Table 3, the phenotypes of the isolates from these four individuals obtained either by cocultivation with PBL or MDM are summarized. In the PBL isolation procedure, a decrease in the time before detection of HIV-1 replication was observed, reflecting the emergence of more virulent variants (33, 35). In contrast, significant changes in the replication rate of HIV-1 isolates in MDM culture were not observed. Except for the last sample from individual 105,

which induced few, small syncytia, all MDM-derived isolates were NSI in PBL, even when the PBL-derived isolate from the same sample was SI, and none of them could be transmitted to T-cell lines.

These results demonstrate that virus isolation by coculture with MDM preferentially rescues HIV-1 variants with an NSI phenotype in PBL culture even when SI clones, which have a growth advantage in PBL culture (32), are present in the same patient sample. This again indicates that monocytotropic variants reside mainly in the population of NSI, non-T-cell-line-tropic HIV-1 clones.

DISCUSSION

In previous studies, we demonstrated that HIV-1 isolates may be divided into two groups, SI and NSI, according to their biological phenotype in PBL culture. Also, a correlation between the phenotype of the virus isolate and clinical progression was observed. In this report, we demonstrate an inverse correlation between the SI capacity of HIV-1 isolates and their monocytotropism. In cell-free infection experiments, the majority of NSI isolates (13 of 17) but a minority of SI isolates (4 of 14) were able to productively infect MDM. In addition, NSI and SI variants also varied in the number of MDM from different donors that they could infect (median, three of eight for NSI variants and zero of eight for SI variants). We were not able to demonstrate the presence of intracellular p24 in MDM cultures incubated with SI variants that did not show p24 in the supernatant,

excluding the possibility of intracellular sequestration of virus particles. Low-grade infection is further excluded by our inability to rescue virus from MDM cultures negative for p24 by cocultivation with PBL. It appeared that MDM from different blood donors reproducibly varied in their susceptibility to HIV-1 infection. Only MDM that could be infected by most of the NSI variants could be infected by an SI isolate, indicating that host-determined factors play a role in productive HIV-1 infection of monocytes. The ability of HIV-1 variants with the NSI phenotype to replicate in MDM was confirmed in cell-free infection experiments with biological clones with distinct biological properties, obtained from PBMC from an HIV-1-infected individual by clonal isolation at two time points.

In contrast to the results of Perno et al. (27), in our study pretreatment of MDM cultures with GM-CSF did not enhance infection of monocytes with SI variants, nor did it result in a general increase in expression of monocytopathic NSI variants. For MDM from some donors, virus variants were expressed only in GM-CSF-treated MDM, whereas others were expressed only in untreated MDM. From our data we conclude that the effect of GM-CSF is not virus dependent but may be determined by the donor MDM.

When PHA-PBL and MDM were used as target cells in parallel virus isolation experiments, in MDM cocultures NSI isolates were obtained at all time points but one, even when SI isolates were obtained from the same sample by coculture with PBL. The preferential selection of NSI variants and the inefficiency of SI variant rescue by this method confirm the monocytopathism of NSI variants as opposed to that of SI variants.

Although most investigators agree that monocytopathic HIV-1 isolates are unable to replicate in T-cell lines (5, 6, 28), apparently conflicting results have been obtained for the cytopathic effects of HIV-1 infection in monocyte cultures. Formation of multinucleated giant cells has been observed with some isolates (16). Others, however, have drawn attention to the relative absence of cytopathic changes in HIV-1-infected monocyte cultures in comparison to infected PBL cultures (14, 19, 25, 31). In our experiments, morphological changes related to differentiation of monocytes following adherence were noticed, the extent of which seemed to be mainly donor dependent. However, we failed to observe specific cytopathic changes related to HIV-1 infection of monocytes with either NSI or SI variants.

In this study, primary isolates were obtained from peripheral blood from asymptomatic individuals. Others studying HIV-1 monocytopathism have used mainly tissue-derived isolates (e.g., lung and brain), mostly obtained from patients with AIDS (5, 14, 16). Therefore, it may be that cytopathic properties in monocyte culture are confined to certain HIV-1 isolates found in tissues, possibly only from individuals in late stages of disease.

In a recent study by Cheng-Mayer et al. (5), none of the established isolates obtained from peripheral blood were able to infect monocytes, in contrast to those obtained from cerebrospinal fluid. However, all the blood-derived isolates in that study except one were derived from individuals with AIDS-related complex or AIDS and were T-cell-line-tropic (3). The results of our study demonstrate that monocytopathism is not an exclusive characteristic of neurotropic HIV-1 variants, although our finding that NSI isolates from patients with AIDS-related dementia are among the best monocytopathic isolates fits with a role for highly monocytopathic variants in HIV-1-related neurological disease.

Although as a rule monocytopathic isolates in this study

were NSI in PBL culture, four SI isolates established by PBL coculture were able to infect monocytes to some extent and retained their SI capacity in PBL upon passage through MDM, indicating monocytopathism of at least some of the SI clones present (Fig. 1). These isolates (with the possible exception of HTLV-IIIB) were all derived from symptomatic individuals in whom SI variants had been present for some time (33). Similarly, in our primary isolation studies on MDM, the only isolate able to induce syncytia to some extent in PBL culture was recovered from the last sample from subject 105, in whom SI isolates were detected by PBL coculture at least 1.5 years before. This indicates that monocytopathism may occur in some late-stage SI clones. Similar observations have been made by Cheng-Mayer et al. (5). Previously we described increasing tropism of SI isolates for the U937 cell line with ongoing disease (33), again indicating further evolution of the SI phenotype in late stages of infection.

What might be the role of monocytopathic NSI variants in the pathogenesis of HIV-1 infection? Previously we showed that early after seroconversion, only NSI variants are observed (33), as was the case for the seven individuals with known times of seroconversion studied here. This suggests that upon transmission, SI variants which may have been present in the inoculum are eliminated in the recipient by the, at that time, uncompromised immune system. This phenomenon indeed has been reported in perinatal transmission studies, in which the virus isolate from the child of a mother from whom SI variants were recovered appeared to become NSI or much more monocytopathic (2, 25). Further support for this hypothesis comes from the case of a laboratory worker who accidentally got infected with the T-cell-line-tropic HTLV-IIIB isolate and from whom only a genetically similar monocytopathic variant could be isolated (40).

A role for macrophages as a virus reservoir in HIV-1 infection has been postulated previously (21, 41). Our finding that monocytopathic variants can be detected throughout HIV-1 infection corroborates this notion. The low replication rate and NSI phenotype in PBL culture of these isolates and the absence of cytopathogenicity in monocyte culture, together with the intracellular sequestration reported previously (26), may reflect low expression and low immunogenicity of these HIV-1 variants *in vivo*. Apparently, these variants are optimally adapted for survival in early HIV-1 infection, when the anti-HIV-1 immune response is thought to be most effective (20, 37). Therefore, these variants may be of crucial significance for the persistence of HIV-1 infection in asymptomatic individuals. It remains to be investigated to what extent these isolates are directly responsible for the qualitative immune dysfunction preceding the emergence of more virulent HIV-1 variants and overt CD4⁺ cell decline (17, 38).

The monocytopathism of NSI variants and the general absence of monocytopathism in the SI variant population indicate that NSI variants are not just functionally impaired HIV-1 mutants, but may be endowed with specific properties lacking in the otherwise more efficiently replicating SI variants. Preliminary studies with monocytopathic and non-monocytopathic molecular clones generated in our laboratory (16a) indicate that monocytopathism is determined at the level of entry, suggesting involvement of the *env* gene. More detailed molecular and mechanistic studies will be needed to define precisely the determinants for monocytopathism and the cause of the apparent linkage between this property and the inability to induce syncytia in primary T cells.

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